The Chittagong Univ. J. B. Sci.,Vol. 6(1 &2):27-39, 2011.

TOXIC RESPONSE OF THREE PREDATORY FISHES TO FOUR INDEGENOUS PLANT SEED EXTRACTS

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ABSTRACT

Toxicity of seed extracts of four indigenous plants, *Luffa acutangula* (Roxb.), *Areca catechue* (Linn.), *Brassica nigra* (Linn. Koch.) and *Brassica hirta* (Monech) was tested on three predatory fishes- *Heteropneustes fossilis* (Bloch), *Anabas testudineus* (Bloch) and *Channa punctatus* (Bloch) under normal laboratory conditions. Due to the toxic effect of different seed extracts and concentrations, mortality rate varied. It also varied from species to species. On the basis of LC_{50} values of absolute ethyl alcohol extracts, *L. acutangula* seed extracts were more toxic for *H. fossilis* and *A. testudineus*. *A. catechue* seed extracts were of medium toxicity for all the experimental fishes. *B. nigra* seed extracts were less toxic whereas *B. hirta* seed extracts showed differential toxicity being more toxic for *C. punctatus*, medium for *A. testudineus* and less toxic for *H. fossilis*. The susceptibility pattern also varied in the three fish species. In case of *L. acutangula*, *A. catechue* and *B. nigra* seed extracts, the susceptibility was in the same order: *H. fossilis* > *A. testudineus* > *C. punctatus* but the effect of *B. hirta* seed extracts was reverse. To determine the ichthyotoxicity of the four seed extracts on the three predatory fishes, the present study was undertaken.

Key words: Ichthyotoxicity, Plant extracts, Mortality, LC_{50}, Susceptibility.

INTRODUCTION

Presence of undesirable predatory fishes decreases fish production. To increase production, eradication of undesirable fishes from culture ponds is an essential preliminary step. The methods commonly applied to eradicate predatory and weed fishes mainly include chemical pesticides. Majority of the chemical pesticides are highly toxic to fishes and other aquatic organisms. The indiscriminate use of these pesticides may pose a serious threat to our open water capture and flood plain fisheries (Alam *et al*. 1994).

Fish poisons from plant derivated toxicants are accepted for their biodegradable characters and environmental friendliness. The chief products of

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plant derivatives, such as, nicotine, saponin, sabadilla, quassia, ryania and pyrethrum are used in controlling predatory and weed fishes. Chopra *et al*. (1985) mentioned a considerable number of indigenous plant species for use in piscicidal work. Fruit of *Pyrus pashia* and *Croton tiglium*, powdered root of *Mallitia pachycarpa* are also found to be effective for fish (Bhuiyan 1967). Powder from the roots of *Balantes roxburghii*, seeds of *Randia dumertorum*, twigs and roots of *Derris elleptica* and barks of *Albizia lebbeck* had piscicidal effects on the fingerlings of *Cyprinus carpio communis* (Shirgur 1975). Recently, toxic effect of plant extracts have been reported by Tiwari and Singh (2003), Cagauan *et al*. (2004), Usman *et al*. (2005) and Jothivel and Paul (2008).

A good number of indigenous plants is used in piscicidal work in Bangladesh. Such works have been carried out by Ameen and Shahjahan (1987), Ameen *et al*. (1987), Latifa *et al*. (1987, 1988, 1992, 1997, 2002, 2004, 2006), Latifa and Begum (1993), Nasiruddin *et al*. (1997, 1998, 2006, 2009) and Nasiruddin and Sultana (2007).

By using plant originated piscicides as a substitution of chemical pesticides to control the undesirable fishes safely, the rapidly growing environmental pollution could be reduced. From this point of view, search for an ideal fish toxicant from indigenous plant sources could explore a new horizon to the field of fishery management. Evidently, there is a need for evolving piscicides of plant origin from using our vast natural resources for safe removal of undesirable species to ensure protection to useful aquatic lives. In view of these considerations, the assaying of piscicidal activity in terms of toxicity of some indigenous plants was initiated. The present study was carried out with the assaying of extracts of four indigenous plant seeds: *Luffa acutangula* (Roxb.), *Areca catechue* (Linn.), *Brassica nigra* (Linn. Koch.) and *Brassica hirta* (Monech.), on three predatory fish species: *Heteropneustes fossilis* (Bloch), *Anabas testudineus* (Bloch) and *Channa punctatus* (Bloch) to evaluate the toxicity and determine the relative tolerance to these toxicants.

MATERIALS AND METHODS

During the bioassay different sets of the experimental fishes were collected from local markets of Chittagong city on the days of the experiments. Collected fishes were brought immediately to the laboratory and acclimatized for 3-4 hours in laboratory condition. The average total length and weight of the fishes used in the experiments were respectively 11.20 $\pm$ 0.46 cm and 8.55 $\pm$ 0.87g for *H. fossilis*, 11.47 $\pm$ 0.47 cm and 41.35 $\pm$ 1.86 g for *A. testudineus* and 12.75 $\pm$ 0.53 cm and 26.36 $\pm$ 2.3 g for *C. punctatus.*

To extract the toxicants, the seeds were pulverized into fine powder separately in a mortar and then in an electric grinder and then sieved through a 0.025 cm^2 mesh size sieve. The required amount of powder was mixed with required volume of distilled water, 50% or absolute ethyl alcohol separately. To ensure complete extraction, the flask was shaken vigorously in a magnetic stirrer for 3-4 hours. The resultant liquid was filtered through four folds of a fine muslin cloth. The filtrate was the stock solution and the different concentrations (ppm) were prepared from the stock solution by appropriate dilution (APHA 1976).

Before the final experiments, many preliminary screenings were conducted on trial and error basis to determine the doses resulting 10-90% mortalities. Each experimental aquarium contained 5-litre of tap water and toxicant. Five concentrations of each extract were used. In each test, five test fishes were released in each concentration and each dose of the relevant toxicant was replicated twice and duration of the exposure period was 24 hours. In each set of experiment a control set was similarly maintained in non-chlorinated tap water. Mortality (%) was counted only with those fishes, which died within 24 hours after treatment. The temperature (^{o}C), pH and dissolved oxygen (DO) readings of water of control and experimental aquaria were recorded before the start and at the end of the experiments using ^{o}C thermometer, pH meter and portable DO meter. There was no mortality in the control sets of experiments.

Following the methods of Finney (1971) mortality data were subjected to probit analysis. With 95% confidence limits the values of LC_{50} were analysed using a computer based probit analysis programme. Chi-square (χ^2) values were determined (Fisher and Yates 1963) and compared at 0.05 level of significance. Analysis of variance of mortality of fishes was made to estimate the variation among treatments at 0.01 level of significance. The relative potencies of equitoxic toxicants were calculated by taking the highest LC_{50} values of the toxicant as unit and comparing with the respective LC_{50} values of other toxicants.

RESULTS AND DISCUSSION

At 24 hours exposure mortalities of the three test fishes with the four experimental plant seed extracts have been shown in Table-1.

TABLE 1: SHOWING % MORTALITIES OF THE THREE TEST FISHES IN DIFFERENT CONCENTRATIONS OF THE FOUR EXPERIMENTAL SEED EXTRACTS.

Seed	Extract	*Heteropneustes fossilis*		*Anabas testudineus*		*Channa punctatus*	
		Conc. (ppm)	Mortality (%)	Conc. (ppm)	Mortality (%)	Conc. (ppm)	Mortality (%)
Luffa acutangula	Distilled water	50	20	50	20	250	20
		75	50	100	40	500	40
		100	70	150	60	750	60
		200	80	200	80	1000	80
		300	90	250	90	1250	90
	50% ethyl alcohol	25	20	25	10	100	20
		50	40	50	40	250	40
		75	60	100	60	350	50
		100	70	150	70	500	70
		200	90	200	90	750	80
	Absolute ethyl alcohol	5	10	10	20	50	10
		10	30	25	30	100	30
		25	60	50	50	250	50
		50	70	100	70	350	60
		75	80	150	90	500	80
Areca catechue	Distilled water	50	10	50	10	100	10
		100	30	100	30	150	30
		250	50	200	50	250	50
		350	70	300	60	350	70
		500	90	400	80	450	90
	50% ethyl alcohol	25	20	50	20	100	20
		50	30	75	40	150	40
		100	50	150	60	200	60
		250	70	225	70	250	70
		350	90	300	90	300	90
	Absolute ethyl alcohol	10	20	25	10	50	20
		25	40	50	30	100	40
		50	60	100	60	150	60
		100	80	175	70	200	70
		150	90	250	90	250	80
Brassica nigra	Distilled water	100	20	300	20	500	20
		250	50	400	40	750	50
		400	60	500	60	1000	60
		550	70	600	70	1250	80
		700	90	700	80	1500	90
	50% ethyl alcohol	75	20	50	10	250	20
		150	40	100	30	350	30
		250	50	200	50	500	50
		300	70	300	70	750	70
		400	80	400	80	1000	90
	Absolute ethyl alcohol	25	20	50	20	150	20
		50	40	100	30	300	50
		100	60	200	60	450	60
		250	80	275	70	600	80
		400	90	350	90	750	90
Brassica hirta	Distilled water	100	10	50	10	25	10
		200	30	100	30	50	20
		300	50	200	50	100	40
		400	60	300	70	200	60
		500	80	400	90	400	90
	50% ethyl alcohol	50	20	25	10	25	10
		100	30	50	30	50	30
		200	50	100	50	100	50
		300	70	200	70	200	70
		400	90	300	90	300	90
	Absolute ethyl alcohol	25	20	25	20	25	20
		50	30	50	30	50	40
		100	50	100	50	100	60
		200	60	150	70	175	70
		300	80	200	90	200	90

The regression equation obtained from probit analysis, the chi-square values at 0.05 level and the F values at 0.01 level, the LC_{50} values with confidence limits have been given in Table-2. The relative potencies of distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts of *L. acutangula*, *A. catechue*, *B. nigra* and *B. hirta* seeds on *H. fossilis*, *A. testudineus* and *C. punctatus* have been presented in Table-3, whereas ranking of the relative potency values of the extracts and their toxicity categories have also been shown in Table-2.

Dominance hierarchy of the experimental plant seed extracts

The dose ranges, LC_{50} and relative potency values of absolute ethyl alcohol seed extracts of the experimental plant seeds on the three test fishes have been given in Table-3. From the table it is observed that for fish the order of toxicity is in the order: In case of:
H. fossilis – L. acutangula > *A. catechue* > *B. nigra* > *B. hirta* seed extracts, *A. testudineus – L. acutangula* > *B. hirta* > *A. catechue* > *B. nigra* seed extracts, and *C. punctatus* - *B. hirta* > *A. catechue* > *L. acutangula* > *B. nigra* seed extracts.
L. acutangula seed extracts were most toxic for *H. fossilis* and *A. testudineus* whereas *A. catechue* seed extracts were of medium toxicity for all the experimental fishes. *B. nigra* seed extracts was found to be less toxic. However, *B. hirta* seed extracts showed differential toxicity as it was most toxic with *C. punctatus*, medium with *A. testudineus* and less toxic with *H. fossilis*.

Towards plant seed extracts sensitivity of the fishes was in the order:
In case of *L. acutangula* seed extracts – *H. fossilis* > *A. testudineus* > *C. punctatus;* for *A. catechue* seed extracts - *H. fossilis* > *A. testudineus* > *C. punctatus*; for *B. nigra* seed extracts - *H. fossilis* > *A. testudineus* > *C. punctatus;* and in case of *B. hirta* seed extracts - *C. punctatus* > *A. testudineus* > *H. fossilis*. The toxicity of *L. acutangula*, *A. catechue* and *B. nigra* seed extracts was in the same trend for *H. fossilis*, *A. testudineus* and *C. punctatus* but the effect of *B. hirta* seed extracts was vice versa i.e., being most effective for *C. punctatus* and less effective for *H. fossilis*. The effectivity for *A. testudineus* was in the same order in all the three plant extracts.

TABLE 2: TOXICITIES AND STATISTICAL ANALYSIS OF THE VALUES OF THE FOUR EXPERIMENTAL SEED EXTRACTS ON THE THREE TEST FISHES EXPOSED FOR 24 HOURS.

Fish	Seed	Extract	Regression equation	χ^2-test		F-test		LC_{50} (ppm)	Conf. limit (Lower)	Conf. limit (Upper)	Relative potency	Ranking of relative potency *
				value	p	value	p					
H. fossilis	*Luffa acutangula*	Dist. water	0.13+2.53x	10.94	p<0.05	12.83	p<0.01	82.528	45.061	118.824	3.572	Medium toxic
		50% eth. alc.	0.78+2.38x	0.60	p>0.05	18.25	p<0.01	59.647	34.679	89.323	4.942	Medium toxic
		Abs. eth. alc.	2.68+1.71x	3.50	p>0.05	14.17	p<0.01	22.158	11.867	40.605	13.301	Most toxic
	Areca catechue	Dist. water	-0.14+2.25x	9.08	p>0.05	10.00	p>0.01	191.295	117.360	298.886	1.541	Less toxic
		50% eth. alc.	1.85+1.60x	6.54	p>0.05	10.25	p>0.01	93.479	48.066	170.162	3.153	Medium toxic
		Abs. eth. alc.	2.34+1.76x	1.01	p>0.05	41.00	p<0.01	32.882	15.610	55.539	8.964	Highly toxic
	Brassica nigra	Dist. Water	-0.20+2.15x	6.04	p>0.05	11.17	p>0.01	260.453	127.767	396.203	1.132	Less toxic
		50% eth. alc.	-0.15+2.25x	5.74	p>0.05	14.25	p<0.01	193.333	107.110	307.173	1.525	Less toxic
		Abs. eth. alc.	1.78+1.72x	0.68	p>0.05	41.00	p<0.01	73.792	34.888	128.341	3.994	Medium toxic
	Brassica hirta	Dist. water	-2.28+2.94x	2.59	p>0.05	12.17	p<0.01	294.724	205.614	438.110	1.000	Least toxic
		50% eth. alc.	0.82+1.90x	12.14	p<0.05	10.25	p>0.01	155.868	90.120	247.627	1.891	Less toxic
		Abs. eth. alc.	2.04+1.47x	2.80	p>0.05	43.85	p<0.01	104.075	50.019	237.711	2.832	Less toxic
A. testudineus	*Luffa acutangula*	Dist. water	-1.11+2.99x	6.17	p>0.05	41.00	p<0.01	109.061	68.156	148.476	4.161	Medium toxic
		50% eth. alc.	0.43+2.43x	7.39	p>0.05	15.50	p<0.01	74.793	46.037	111.059	6.067	Highly toxic
		Abs. eth. alc.	2.23+1.71x	8.41	p>0.05	10.25	p>0.01	41.924	21.202	75.467	10.824	Most toxic
	Areca catechue	Dist. water	0.12+2.13x	2.95	p>0.05	12.17	p<0.01	192.558	119.454	330.666	2.357	Less toxic
		50% eth. alc.	0.26+2.31x	5.88	p>0.05	18.25	p<0.01	109.423	62.817	164.341	4.147	Medium toxic
		Abs. eth. alc.	0.47+2.35x	5.06	p>0.05	12.75	p<0.01	85.008	52.909	129.949	5.338	Medium toxic
	Brassica nigra	Dist. water	-6.55+4.33x	1.26	p>0.05	29.00	p<0.01	453.757	338.337	562.653	1.000	Least toxic
		50% eth. alc.	-0.16+2.29x	0.92	p>0.05	10.25	p>0.01	180.137	113.328	287.270	2.519	Less toxic
		Abs. eth. alc.	0.057+2.30x	10.44	p<0.05	13.84	p<0.01	141.552	82.719	214.100	3.206	Medium toxic
	Brassica hirta	Dist. water	-0.71+2.56x	6.65	p>0.05	10.00	p>0.01	169.119	109.835	250.738	2.684	Less toxic
		50% eth. alc.	0.69+2.17x	3.18	p>0.05	10.00	p>0.01	95.802	58.334	154.589	4.737	Medium toxic
		Abs. eth. alc.	0.94+2.13x	12.49	p<0.05	10.25	p>0.01	77.934	45.060	123.814	5.823	Medium toxic
C. punctatus	*Luffa acutangula*	Dist. water	-3.27+3.01x	5.29	p>0.05	41.00	p<0.01	545.303	340.779	742.382	1.452	Less toxic
		50% eth. alc.	0.16+1.95x	3.26	p>0.05	14.25	p<0.01	302.272	155.734	511.170	2.620	Less toxic
		Abs. eth. alc.	0.61+1.87x	3.64	p>0.05	12.17	p<0.01	221.356	129.283	406.775	3.577	Medium toxic
	Areca catechue	Dist. water	-3.27+3.50x	4.67	p>0.05	10.00	p>0.01	228.911	168.470	306.042	3.459	Medium toxic
		50% eth. alc.	-4.35+4.18x	4.51	p>0.05	18.25	p<0.01	169.198	122.061	212.999	4.680	Medium toxic
		Abs. eth. alc.	0.65+2.08x	1.66	p>0.05	29.00	p<0.01	118.352	66.663	177.176	6.690	Highly toxic
	Brassica nigra	Dist. water	-7.59+4.33x	2.99	p>0.05	18.75	p<0.01	791.753	559.266	982.541	1.000	Least toxic
		50% eth. alc.	-4.07+3.73x	4.25	p>0.05	10.25	p>0.01	482.043	346.697	651.480	1.643	Less toxic
		Abs. eth. alc.	-2.14+2.86x	4.80	p>0.05	18.75	p<0.01	309.303	182.366	424.636	2.560	Less toxic
	Brassica hirta	Dist. water	0.65+2.07x	5.14	p>0.05	25.75	p<0.01	125.459	77.413	221.066	6.311	Highly toxic
		50% eth. alc.	0.72+2.17x	3.79	p>0.05	10.00	p>0.01	95.802	58.334	154.589	8.265	Highly toxic
		Abs. eth. alc.	1.50+1.88x	5.69	p>0.05	18.25	p<0.01	71.443	35.811	117.920	11.083	Most toxic

*Rank assigned to relative potency values: Most toxic > 10.00, Highly toxic > 6.00 to < 10, Medium toxic > 3.00 to < 6.00, Less toxic > 1.00 to < 3.00 and Least - 1.00 and below.

TABLE 3: SHOWING THE DOSE RANGES, LC_{50} AND RELATIVE POTENCY VALUES OF THE ABSOLUTE ETHYL ALCOHOL EXTRACTS OF THE FOUR EXPERIMENTAL SEEDS ON THE THREE TEST FISH.

Seed	Fish	Dose range (ppm)	LC_{50} (ppm)	Relative potency
Luffa acutangula	*Heteropneustes fossilis*	5 - 75	22.158	13.301
	Anabus testudineus	10 – 150	41.924	10.824
	Channa punctatus	50 – 500	221.356	3.577
Areca catechue	*Heteropneustes fossilis*	10 – 150	32.882	8.964
	Anabus testudineus	25 -250	85.008	5.338
	Channa punctatus	50 – 300	118.352	6.690

Brassica nigra	*Heteropneustes fossilis*	25 – 400	73.792	3.994
	Anabus testudineus	50 – 350	141.552	3.206
	Channa punctatus	350 – 750	309.303	2.506
Brassica hirta	*Heteropneustes fossilis*	25 – 300	104.075	2.832
	Anabus testudineus	25 – 200	77.934	5.823
	Channa punctatus	25 - 250	71.443	11.083

Effect of the plant seed extracts on temperature, pH and DO values of water of the experimental aquaria

The mean of temperature, pH and dissolve oxygen (DO) values of the water of the experimental doses before and after the experiments have been shown in Table 4. In almost every case the temperature values in all the three extracts of the seeds of the four indigenous plants decreased slightly after the experiments. The pH values also decreased slightly at the end of the experiments but were in the acidic range. In case of DO, the values decreased tremendously after 24 hours of exposure. For *H. fossilis, A. testudineus* and *C. punctatus* the DO ranged from 0.12-0.86, 0.18-1.62 and 0.26-1.32 mg/l respectively after the experiments.

TABLE 4: MEAN AND ±SE OF TEMPERATURE, pH AND DISSOLVED OXYGEN OF WATER OF THE EXPERIMENTAL AQUARIA TREATED WITH THE FOUR EXPERIMENTAL SEED EXTRACTS ON THE THREE TEST FISH EXPOSED FOR 24 HOURS.

Fish	Seed	Extract	Temperature (^{0}C)		pH		Dissolved Oxygen (mg/l)	
			Before expt.	After expt.	Before expt.	After expt.	Before expt.	After expt.
H. fossilis	*Luffa acutangula*	Dist. water	30.92±0.10	29.90±0.07	7.06±0.05	6.86±0.05	3.66±0.05	0.24±0.02
		50% et. alc.	22.18±0.08	23.22±0.09	7.04±0.05	6.70±0.03	3.28±0.04	0.14±0.02
		Abs. et. alc.	29.28±0.09	30.06±0.10	6.72±0.04	6.62±0.04	3.16±0.05	0.12±0.02
	Areca catechue	Dist. water	31.08±0.07	30.10±0.07	6.90±0.03	6.76±0.05	3.68±0.04	0.86±0.05
		50% et. alc.	22.04±0.09	23.30±0.07	6.54±0.05	6.56±0.08	3.18±0.06	0.44±0.02
		Abs. et. alc.	29.34±0.09	29.28±0.09	6.34±0.07	6.40±0.07	3.10±0.03	0.18±0.04
	Brassica nigra	Dist. water	26.14±0.05	26.16±0.07	6.74±0.05	6.78±0.04	3.54±0.05	0..36±0.02
		50% et. alc.	22.08±0.04	23.14±0.05	6.60±0.07	6.58±0.04	3.48±0.06	0.30±0.07
		Abs. et. alc.	24.10±0.03	23.08±0.04	6.40±0.03	6.44±0.05	3.20±0.07	0.16±0.04
	Brassica hirta	Dist. water	29.00±0.07	28.04±0.05	7.04±0.05	6.94±0.05	3.66±0.04	0.46±0.02
		50% et. alc.	24.98±0.08	27.08±0.06	6.80±0.07	6.92±0.06	3.62±0.07	0.44±0.04

		Abs. et. alc.	24.10±0.07	23.02±0.07	6.66±0.05	6.70±0.07	3.22±0.04	0.18±0.04
A. testudineus	*Luffa acutangula*	Dist. water	24.02±0.04	22.62±0.06	7.16±0.05	6.80±0.05	6.62±0.06	0.66±0.02
		50% et. alc.	23.06±0.02	22.04±0.05	6.80±0.05	6.72±0.04	6.18±0.04	0.64±0.04
		Abs. et. alc.	23.08±0.04	23.04±0.05	6.62±0.04	6.36±0.05	4.80±0.03	0.30±0.03
	Areca catechue	Dist. water	24.56±0.05	21.00±0.08	7.08±0.04	6.78±0.06	6.86±0.05	1.62±0.04
		50% et. alc.	23.02±0.04	22.28±0.07	6.86±0.05	6.76±0.04	6.40±0.07	0.36±0.02
		Abs. et. alc.	23.46±0.05	22.02±0.04	6.70±0.05	6.56±0.05	4.96±0.05	0.30±0.03
	Brassica nigra	Dist. water	24.44±0.07	23.04±0.05	6.76±0.05	6.46±0.05	6.56±0.05	0.80±0.03
		50% et. alc.	23.52±0.07	22.06±0.05	6.68±0.06	7.06±0.04	6.30±0.03	0.22±0.04
		Abs. et. alc.	22.04±0.02	20.98±0.04	6.66±0.05	6.70±0.03	4.92±0.04	0.18±0.04
	Brassica hirta	Dist. water	24.04±0.05	22.08±0.06	6.88±0.04	7.04±0.05	6.74±0.05	1.08±0.04
		50% et. alc.	24.02±0.08	24.04±0.09	6.64±0.05	6.90±0.07	6.40±0.05	0.26±0.02
		Abs. et. alc.	22.10±0.03	23.02±0.07	6.58±0.04	6.62±0.04	4.94±0.05	0.18±0.04
C. punctatus	*Luffa acutangula*	Dist. water	21.00±0.05	20.44±0.02	7.02±0.04	7.08±0.04	6.16±0.02	0.60±0.05
		50% et. alc.	22.20±0.03	19.96±0.05	6.80±0.03	6.76±0.05	5.80±0.05	0.34±0.04
		Abs. et. alc.	21.04±0.05	19.08±0.06	6.78±0.06	6.74±0.05	3.82±0.04	0.26±0.02
	Areca catechue	Dist. water	24.34±0.05	23.04±0.05	6.64±0.05	6.64±0.05	6.80±0.05	1.16±0.05
		50% et. alc.	22.02±0.04	21.14±0.06	6.68±0.06	6.58±0.07	6.18±0.06	0.40±0.05
		Abs. et. alc.	19.96±0.05	19.12±0.06	6.38±0.06	6.24±0.05	4.20±0.03	0.32±0.04
	Brassica nigra	Dist. water	23.14±0.05	21.42±0.04	6.80±0.05	6.48±0.06	6.40±0.03	1.26±0.05
		50% et. alc.	22.14±0.05	21.12±0.06	6.72±0.04	6.46±0.08	6.08±0.04	0.58±0.06
		Abs. et. alc.	21.12±0.06	18.36±0.05	6.38±0.06	6.28±0.06	3.96±0.05	0.42±0.04
	Brassica hirta	Dist. water	22.98±0.04	23.96±0.05	6.68±0.04	6.78±0.04	6.10±0.03	1.32±0.06
		50% et. alc.	23.04±0.05	21.06±0.05	6.56±0.05	6.50±0.05	5.98±0.06	0.52±0.04
		Abs. et. alc.	19.96±0.05	19.42±0.04	6.44±0.08	6.46±0.05	3.96±0.05	0.30±0.03

Bioassay experiment is necessary for the determination of relationship of a particular organism with a particular environment. It can determine the toxicity level of extracts on different species, reaction of different environmental factors in response to the toxicity of water and aquatic animals.

Due to the toxic effects of different seed extracts and concentrations, mortality rate varied in the three fish species. From the study it was realized that a particular concentration of an extract could cause a varying percentage of mortality in the three species of fishes. From the mortality data it was also observed that the mortality of fishes increased with gradual increase of dose concentration of different extracts. Within the concentrations studied the differential mortality ranged from 10-90%. On the basis of LC_{50} values of absolute ethyl alcohol extracts in case of *H. fossilis,* the toxicity of the seed extracts was found to be in the order: *L. acutangula* > *A. catechue* > *B. nigra* > *B. hirta* seed extracts. Whereas in *A. testudineus* the toxicity of the four seed extracts followed the pattern: *L. accutangula* > *B. hirta* > *A. catechue* > *B. nigra* seed extracts. The toxicity of the four seed extracts on *C. punctatus* was: *B. nigra* > *A. catechue* > *L. acutangula* > *B. nigra* seed extracts. *L. acutangula* seed extracts were more toxic for *H. fossilis* and *A. testudineus*. *A. catechue* seed extracts were medium toxic for all the experimental fishes. *B. nigra* seed extracts were less toxic, whereas *B. hirta* seed extracts showed differential toxicity being more toxic in *C. punctatus*, medium in *A. testudineus* and less to *H. fossilis*.

The susceptibility pattern or degree of tolerance varied from species to species. In case of *L. acutangula*, *A. catechue* and *B. nigra* seed extracts, the degree of suceptibility was in the same order i.e., *H. fossilis* > *A. testudineus* > *C. punctatus* but the effect of *B. hirta* seed extracts was vice versa, i.e., being more effective in *C. punctatus* and less effective in *H. fossilis*.

Observing the relative potency values of the absolute ethyl alcohol extracts, in case *H. fossilis* the comparative relative potencies of the experimental seeds were observed in the order: *L. acutangula* (13.302) > *A. catechue* (8.964) > *B. nigra* (3.994) > *B. hirta* (2.832) seed extracts. In *A. testudineus* the comparative relative potencies stood as: *L. acutangula* (10.824) > *B. hirta* (5.823) > *A. catechue* (5.338) > *B. nigra* (3.206) seed extracts. And in *C. punctatus* the relative potency followed the trend: *B. hirta* (11.083) > *A. catechue* (6.690) >*L. acutangula* (3.577) > *B. nigra* (2.506) seed extracts.

The χ^2 values in case of *H. fossilis* were found insignificant at 0.05 level in almost all the seed extracts excepting distilled water extract of *L. acutangula* and 50% ethyl alcohol extract of *B. hirta*. Whereas the chi-suqare values of seed extracts on *A. testudineus* was observed to be significant in absolute ethyl alcohol extract of *B. nigra* and absolute ethyl alcohol extract of *B. hirta* seeds. But all other chi- square values of all plant seed extracts were insignificant in *C. punctatus*. The insignificant χ^2 values indicated that there was no significant difference between observed and excepted mortalities.

During the experiments, in almost all experiments temperature decreased after 24 hours of exposure which may be related to day temperature. But in some cases CO_2 increased which might be due to respiration of fishes which might result in rise of water temperature. The pH values of most extracts were found to decrease slightly being in acidic range. It was mainly due to the CO_2 respired by the experimental fishes which formed carbonic acid in water, whereas the rise in pH may be due to the reduction in NTP (Latifa *et al*. 1988). However, after the experiments the DO values in case *H. fossilis* ranged from 0.12-0.86, for *A. testudineus* 0.18-1.62 and for *C. punctatus* form 0.26-3.70 mg/l. The reason for such variation was that *H. fossilis*, being scaleless, decomposed quickly and thus the value decreased rapidly. On the other hand, *.A. testudineus* and *C. punctatus* are scaly fishes, decomposition started sometimes after death and thus the DO values in such experiments were slightly higher.

Determining the ichthyotoxicity of *L. acutangula*, *A. catechue*, *B. nigra* and *B. hirta* seed extracts on *H. fossilis, A. testudineus* and *C. punctatus* and for their prospect as fish toxicant the present study was undertaken. In conclusion, it can be said that laboratory toxicity studies of crude dry powder of toxic plant seeds can give optimal information regarding the spectrum of toxicity of the plant toxicants.

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Manuscript received on 24.10.2010; Accepted on 07.07.2012